NF-κB and PI3K/MAPK pathways are crucial for *Chlorella sorokiniana W-87 (CRYPTO™)*- induced activation and maturation of human monocyte-derived dendritic cell

Nien-Tzu Chou, Chieh-Fang Cheng, Hsin-Chieh Wu, Chin-Pen Lai, Li-Tsen Lin, I-Horng Pan#, Ching-Huai Ko#

Biomedical Technology and Device Research Laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan.

Abstract

Chlorella sorokiniana W-87 **(CS) is an unicellular green alga. The extracts of *Chlorella* have been used as treatments for relieving hypertension and modulating immune response. However, the detailed mechanisms are not clear yet. In this study, we sought to study the molecular mechanisms for the polysaccharide fraction of *Chlorella sorokiniana W-87* - induced immune response. We pulsed dendritic cells (DCs) with CS and found that CS could maturate DCs. CS-maturated DC could activate naïve T cells and stimulate T cells proliferation and IFN-γ secretion. Furthermore, CS activated PI3K and MAPKs signaling pathways in DCs by interacting with TLR4 receptor. These CS-activated signaling pathways could further activate NF-κB and induce IL-12 production in DCs. This study provides molecular mechanisms for CS-induced DCs activation and immune response.**

◆ CS induces phenotypic maturation and IL-12 production of human monocyte-derived DC by activating NF-κB

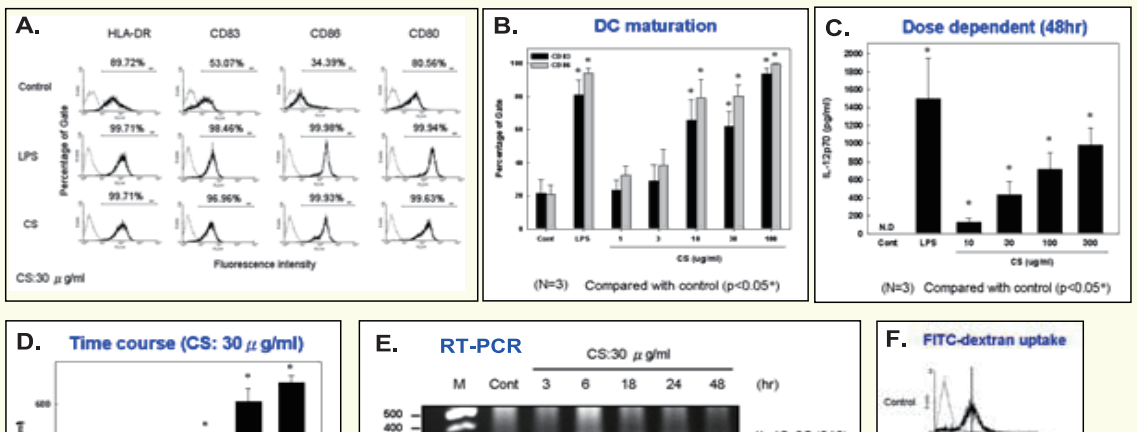

Fig 1. The effects of CS on DC maturation.

(A) Human DCs were treated with CS (30 μg/ml), LPS (1 μg/ml), or medium alone for 48 h, and surface markers were analyzed by flow cytometry (dotted line, isotype control; solid line: specific mAb). The values shown were the percentage of gated cells (Gated %). (B) DCs were treated with CS at different concentrations and the expression of CD83 and CD86 were analyzed by flow cytometry (black bar: CD83; gray bar: CD86). LPS was used as positive control. (C) Human DCs were cultured in the presence of 1 μg/ml LPS or various concentrations of CS for 48 h. IL-12 secretion was analyzed by ELISA after incubation. (D) The time-dependent effect of CS (30 μg/ml) treatments on IL-12 secretion in DCs. IL-12 secretion was analyzed by ELISA. *, $P < 0.05$ compared to control. N.D: nondetectable. (E) RT-PCR analysis of IL-12p35 and IL-12 p40. DCs were incubated in the presence of CS (30 μg/ml) for 3, 6, 18, 24 and 48 h. Representative images of three independent experiments were shown here. Lane M: marker. (F) The effect of CS on DC endocytosis. Human PBMC were cultured for 6 days and monocytes were induced to differentiate to DCs (refer to material and methods section). Immature DCs were stimulated with medium alone, LPS (1 μg/ml), or CS (30 μg/ml) for 48 h, and then incubated with FITC-dextran (0.5 mg/ml) for 1 h at 4℃ (dotted line) or 37℃ (solid line). (G) Human monocyte-derived DC were incubated with CS (30 μg/ml) for the indicated period of time. NF-κB assay was as described in material and methods section. The binding activity of NF-κB was shown as relative OD_{450} levels. LPS (1 μg/ml) treatment for 2 h was used as positive control. *, $P < 0.05$ compared to control.

◆ PI3K/Akt pathway acts upstream of the MAPKs in CS-stimulated DC

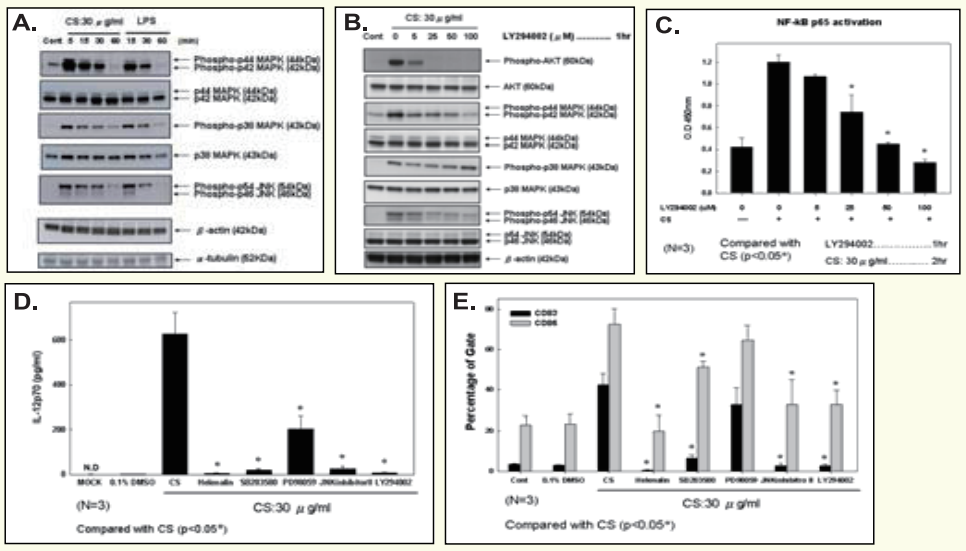

Fig 3. CS induces IL-12 secretion through PI3K/Akt and MAPKs pathways in DCs.

(A). Time-course of p44/42 ERK, p38 MAPK, and p46/54 JNK phosphorylation in CS-stimulated DCs. Human DCs were treated with CS (30 μg/ml) and cell lysates were collected at different time points. (B) Effect of PI3K inhibitor LY294002 on MAPKs in CS-stimulated DCs. Human DCs were pretreated with various concentration of LY294002 (5, 25, 50 and 100 μM) for 1 h prior to CS (30 μg/ml) for 15 min. Cell lysates were collected and MAPK phosphorylations were analyzed by Western blotting (N=3). (C) The PI3K inhibitor LY294002 repressed CS-induced NF-κBp65 binding to DNA. Human DC were pretreated with various concentration of LY294002 (5, 25, 50 and 100 μM) for 1h prior to 2 h stimulation by CS (30 μg/ml). (D, E) Inhibitors against PI3K, NF-κB and MAPKs blocked CS-induced IL-12 secretion (D) and CD83, CD86 up-regulation (E) (black bar: CD83; gray bar: CD86) in DC. Immature DCs were pre-incubated for 1 h with one of the following compounds: LY294002 (25 μM), Helenalin (2.5 μM), SB203580 (20 μM), PD98059 (50 μM), or JNK inhibitor II (20 μM), and followed by CS (30 μg/ml) stimulation for an additional 48 h. *, $P < 0.05$ compared to CS alone. N.D: nondetectable.

◆ CS-treated human DCs Enhance T cell activation

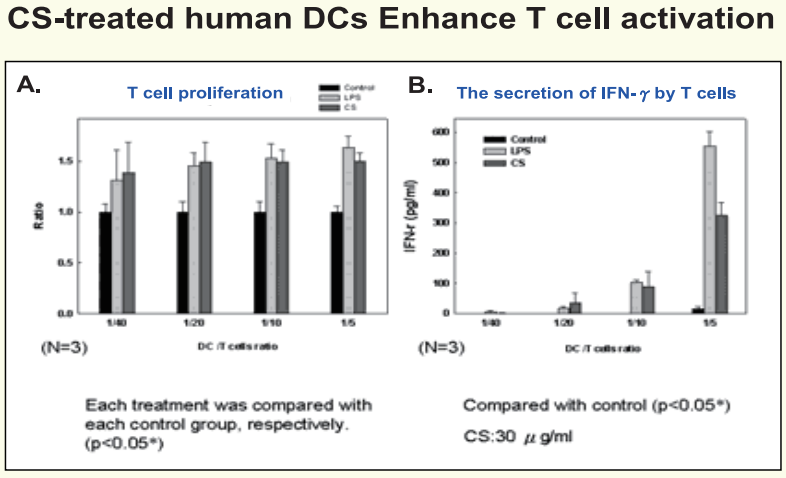

Fig 2. Allogeneic T cell responses induced by CS-treated DCs.

Immature DCs were stimulated with CS (30 μg/ml) or LPS (1 μg/ml) for 48 h. Allogeneic T cells ($1x10^5$) were co-cultured in 96-well U-bottom microplates with CS-treated DCs as described. (A) T cell proliferation was determined by using an Alamar Blue assay 5 days post co-culturing. Experimental groups were compared to corresponding control groups. (B) After 2 days of co-culturing, the production of IFN-γ by T cells was analyzed by ELISA. Data were expressed as means ± SEM of triplicates from three independent experiments. *, $P < 0.05$ compared to control. N.D: nondetectable.

◆ CS-induced TLR4 signaling via the PI3K/Akt –MAPKs pathway

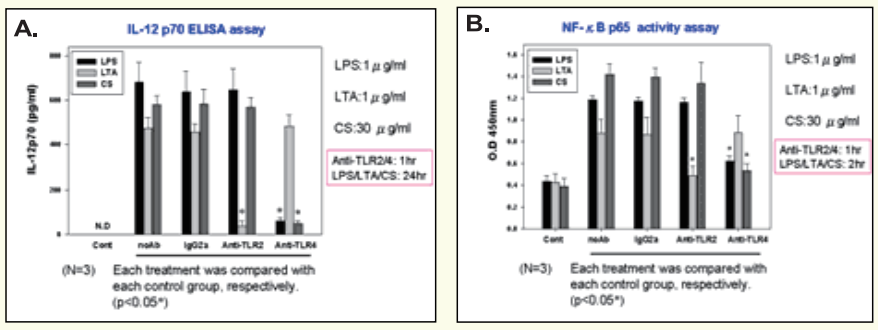

Fig 4. CS induces IL-12 expression through a TLR4-dependent signaling pathway.

(A) Anti-TLR-4 neutralizing antibody blocks CS-induced IL-12 secretion in DCs. DCs were pre-incubated with 20 μg/ml anti-TLR-2, anti-TLR-4, or control IgG for 1 h prior to LPS (1 μg/ml), LTA (1 μg/ml), or CS (30 μg/ml) treatments for 24 h. Conditioned-media were collected for IL-12 detection. (B) NF-κB binding activity was examined in DC treated with CS (30 μg/ml) for 2h with or without the pre-treatments of neutralizing antibody against TLRs or control antibody for 1 h. The effects of neutralizing antibodies treatments were compared to corresponding control IgG treatments. *, $P < 0.05$compared to control antibodies. N.D: non-detectable.

Conclusion

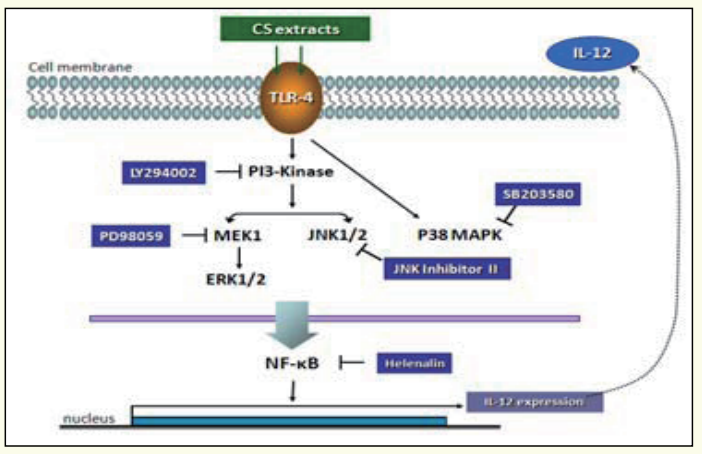

Fig 5. Model of CS-mediated signaling pathways in regulating IL-12p70 expression in DC.

Acknowledgment

Chlorella sorokiniana (W-87) was provided by International Cryptomonadales Biotechnology Co., Ltd. (Taiwan)

Contact information

Corresponding author: I-Horng Pan (e-mail: I-HorngPan@itri.org.tw)
Ching-Huai Ko (e-mail: CHKo@itri.org.tw)